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GRANT NUMBER DAMD17-96-1-6150

TITLE: Control of Cell Migration during Development

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REPORT DATE: August 1998

TYPE OF REPORT: Final

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 1998	Final (1 Aug 96 - 31 Jul 98)

4. TITLE AND SUBTITLE
Control of Cell Migration During Development

5. FUNDING NUMBERS
DAMD17-96-1-6150

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

Cell migrations are critical for normal development. For example, much of the human nervous system is generated by cell migration. Also, the spread of cancerous cells is a similar process. Few proteins that guide migrating cells have been identified. To identify additional proteins required for cell migration, we performed two genetic screens for *Caenorhabditis elegans* mutants with displaced migratory cells. In these screens we identified 14 genes required for normal cell migration. We have cloned two of these gene, *cam-1* and *unc-34*. *cam-1* encodes a receptor tyrosine kinase of the Ror class. Vertebrate Rors are expressed in the nervous system during development, but their function in development is not known. Therefore, our analysis of *cam-1* mutants provides the first indication of Ror function. *unc-34* encodes a homolog of Drosophila *enabled*, a protein implicated in cell migration possible by directly regulating actin polymerization. Therefore, we have identified a gene, *unc-34*, that functions relatively directly in the process of cell migration. Additionally we are investigating the role in cell migration of two other genes identified in our screens. Further characterization of these mutants will shed additional light onto the mechanisms used to guide migrating cells.

14. SUBJECT TERMS
breast cancer
cell migration, genetics, Caenorhabditis, development

15. NUMBER OF PAGES
17

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Wayne Forester July 15, 1998

PI - Signature Date

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INTRODUCTION

Numerous metazoan cell types migrate extensively from their sites of birth to adopt positions critical for normal development. Such cell movements accompany prominent landmarks in early vertebrate development, such as gastrulation and neurulation. In mammals, primordial germ cells move to the gonad, precardiac cells migrate to the heart, and melanocytes migrate to the skin. In addition, the spread of cancer cells is a similar cell migration. My work focuses on the molecular mechanisms and regulation of these migrations, using the cells of the nervous system as a paradigm. In the developing nervous system, neural crest cells move to populate the ganglia of the peripheral nervous system, and neurons migrate to generate the layers of the cerebral cortex. Similarly, neuronal growth cones migrate to their synaptic targets, an essential step in achieving connectivity.

Cell migration is a complex process that requires many proteins. Extracellular matrix components and chemotropic agents function as cues that guide migrating cells along their proper pathways (1) and determine their final destinations (2). Signals from extracellular guidance cues are transmitted by intracellular signaling molecules. Ultimately these signals converge to regulate cell polarity, cell adhesion and the actin cytoskeleton, which together generate the force that drives cell motility (3, 4).

Both biochemical and genetic approaches have been used to identify molecules involved in cell migration. I have chosen to use *Caenorhabditis elegans* as a model genetic system to study cell migration for several reasons. First, *C. elegans* is transparent so it is relatively simple to visually determine the positions of cells that migrate. Additionally, *C. elegans* can easily be transformed to produce transgenic animals. Furthermore, the genomic sequence will be completed by 1998, which provides tools that facilitate the molecular cloning of genes identified by mutation. Finally, because many of the proteins required for cell migration are conserved in mammals, information learned about cell migration in *C. elegans* will be relevant to other organisms.

My ultimate aims are to understand how migrating cells recognize and respond to extracellular guidance cues, and how their migrations are terminated at their proper destinations. My strategy is to identify the molecules involved and to determine their biochemical roles. Specifically, I am identifying genes required for proper cell migration and then using them to identify the genetic circuitry that underlies cell migration. I have identified 14 genes that are required for cell migration in *C. elegans*. Four of the gene products are known to be critical for cell migration in other organisms, demonstrating that the screens I conducted yield proteins that function directly in cell migration. Interestingly, one of the genes I identified, *cam-1*, encodes a receptor tyrosine kinase (RTK) that specifies the final positions of migrating cells and orients cell polarity. *cam-1* is most similar to RTKs of the Ror subclass. Although other Ror proteins are expressed in the developing nervous system (5, 6, 7), my analysis of *cam-1* mutants provides the first evidence that these proteins function in cell migration. A second gene, *unc-34*, encodes an *enabled* (*ena*) homolog. Mutations in Drosophila *ena* result in a disorganized central nervous system, perhaps due to defective growth cone migration (8). The murine homolog, *mena*, has been implicated in cell migration (9). In addition to *cam-1* and *unc-34*, I am investigating the function of two additional genes that I identified in my screens. Analysis of these genes promises to provide additional insights into mechansims of cell migration, neuronal function and development.

BODY

EXPERIMENTAL METHODS, ASSUMPTIONS AND PROCEDURES

Strains and genetics. Strains were grown at 20°C and maintained as described (10) . In addition to the wild-type strain N2, strains with the following mutations were used in this work:

LGI: *unc-73(gm67)*, *unc-73(gm123)*, *cam-2(gm124)*, *syc-3(gm135)*
LGII: *cam-1(gm105)*, *cam-1(gm122)*
LGIII: *ceh-10(gm58)*, *ceh-10(gm71)*, *ceh-10(gm100)*, *ceh-10(gm120)*, *ceh-10(gm127)*, *ceh-10(gm131)*, *ceh-10(gm133)*, *syc-2(gm132)*, *fam-1(gm85)*, *ina-1(gm144)*, *syc-1(gm126)*
LGIV: *epi-1(gm57)*, *epi-1(gm121)*, *epi-1(gm139)*, *epi-1(gm146)*, *fam-2(gm94)*, *kyIs5* [*ceh-23-unc-76-gfp::lin-15*]
LGV: *unc-34(gm104)*, *unc-34(gm114)*, *unc-34(gm115)*, *unc-34(gm134)*, *vab-8(gm99)*, *vab-8(gm138)*
LGX: *mig-2(gm38)*, *mig-2(gm103sd)*, *kyIs4* [*ceh-23-unc-76-gfp::lin-15*]

The isolation and genetic characterization of Cam mutants *epi-1* (epithelialization defective), *ina-1* (integrin, α-subunit), *unc-34* (uncoordinated), *mig-2* (cell migration defective), *unc-73*, *vab-8* (variable abnormal), *ceh-10* (*C. elegans* homeobox), *cam-1*, *cam-2* (CAN abnormal migration), *syc-1*, *syc-2*, *syc-3*, (synthetic Cam), *fam-1* and *fam-2* (fasciculation and cell migration defective) are described by (11). The *ceh-10* alleles *gm71*, *gm100*, *gm131*, and *gm133* all appear identical to *gm58* for all phenotypes examined (11). *kyIs5* is a *ceh-23-gfp* reporter transgene that is integrated on LGIV, and *kyIs4* is the same reporter integrated on LGX (Zallen and Bargmann, personal communication).

Because most of the mutants were isolated in a strain containing the *kyIs5* reporter, we removed this transgene from the mutants by crossing to wild type. The *epi-1* alleles *gm121*, *gm139* and *gm146*, and the *fam-2(gm94)* allele are tightly linked to *kyIs5* and have not been separated from the reporter. For quantitative determinations of CAN axonal morphologies in mutants using green fluorescent protein (GFP) (12), the *kyIs5* reporter was crossed into the mutant backgrounds. For *epi-1(gm57)*, which was isolated in a background lacking *kyIs5*, the *gm57* mutation was crossed into a *kyIs4* background.

Other than in *ceh-10(gm58)* mutants, all cell migrations were scored in homozygous mutant animals derived from homozygous parents. Because *ceh-10(gm58)* is lethal, we examined cell positions in homozygous animals derived from *ceh-10(gm58) / qC1* parents. Because *unc-73(gm67)* and *unc-73(gm123)* progeny of homozygous parents rarely survive to adulthood, we examined axonal morphologies of homozygous mutant animals derived from *unc-73 / dpy-5(e61)* hermaphrodites. Similarly, because *epi-1(gm139)* animals are sterile, we examined axonal morphologies in mutant animals derived from *epi-1(gm139) / dpy-20(e1282*ts*) unc-30(e191)* hermaphrodites.

Scoring of axons and cells. Axons were scored as defective if they deviated from wild-type morphology. This morphology was defined by electron microscopic reconstructions of the *C. elegans* nervous system (13), immunocytochemical staining of wild-type animals (14, 15), and analysis of GFP expression in transgenic animals (16).

The extent of cell migration in wild-type and mutant animals was determined by comparing the positions of nuclei relative to coordinate non-migratory hypodermal nuclei using Nomarski optics. For the CANs, HSNs, ALMs, coelomocyte mother cells, Z1, Z4 and M mesoblast, cells that migrate embryonically, we scored in newly hatched

hermaphrodite larvae the positions of the nuclei of these cells (or their progeny, the coelomocytes, in the case of coelomocyte mother cells) relative to non-migratory hypodermal V and P nuclei. For the Q neuroblasts and their descendants, which migrate during the first larval stage, we scored in late first larval stage hermaphrodites the final positions of the Q descendant nuclei relative to the two daughter hypodermal nuclei derived from V1-6.

V cells were examined by mounting first stage larvae on 5% agar pads on microscope slides in 100 mM sodium azide and examining cells by Nomarski optics microscopy. The Va and Vp cells and nuclei were examined to determine whether their relative positions were reversed in individual animals. Serotonin expressing CAn and CPn neurons were examined by indirect immunofluorescence. Animals were collected, permeabilized, fixed and stained as described (15, 16).

***cam-1* cloning.** To clone the gene, *cam-1* mutations were mapped to a small region of chromosome II, to the right of the cloned gene *let-23* and near or to the left of *mel-11*. Cosmids containing chromosomal DNA from this interval were used to generate transgenic *cam-1(gm122)* animals to determine whether they rescued the mutant phenotypes (17). Deletion of a 6992 bp *Apa*I fragment from one of the rescuing cosmids, C04F7ΔApaI, which rescued *cam-1* mutants, abolished the ability of that cosmid to rescue. Subclones pCAM1 and pCAM2, which contain 20.9 kb *Nhe*I fragment of C25D8 cloned into *Xba*I site of pBluescript KS+ and a 23.3 kb *Pst*I fragment of C25D8 cloned into the *Pst*I site of pBluescript KS+, respectively, were able to rescue *cam-1* mutants. To confirm that the RTK represented *cam-1*, this gene from the mutants was sequenced by the UC Berkeley DNA sequencing facility.

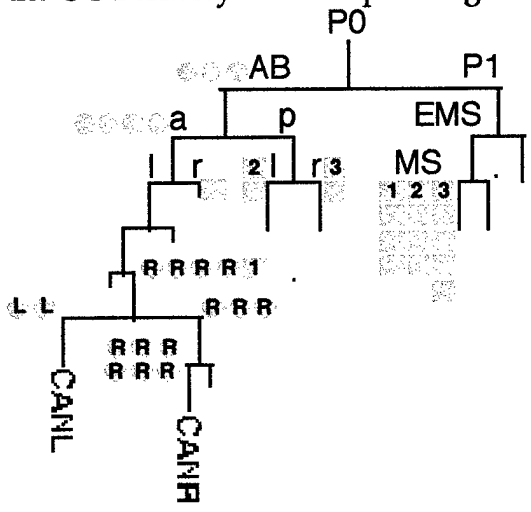

Figure 1. *cam-1* acts cell autonomously in migrating CAN cells. Square indicates loss of the wild type *cam-1* gene that did not result in CAN cell migration defect, and circle indicates a loss that did result in CAN cell migration defect. Numbers in shaded squares indicate that separate losses occurred within two cell lineages. L or R within shaded circle indicates only left or right CAN migration was defective, respectively.

***cam-1* mosaic analysis.** To determine whether *cam-1* functions cell autonomously for CAN cell migration, we performed a mosaic analysis as described (17, 18). Animals of the genotype *cam-1(gm122); ncl-1(e1865); kyIs5[ceh-23-unc-76-gfp::lin-15]; gmEx188[pCAM1::ncl-1(+)::pRF4 rol-6(dm)]* were generated by microinjection (19). Initially, we identified animals in which one or both CAN cells were misplaced and then determined which cells retained the array and which did not. In these animals, 22 CAN cells were misplaced and 21 of these had lost the array as scored by their being Ncl- (figure 1). One of the 22 misplaced CANs was wild type for *ncl-1*, consistent with the observation that rescue of the CAN cell migration defect by *gmEx188* is not 100% (unpublished observation). We identified 20 animals in which some cells were Ncl and others were not and determined whether CANs were misplaced and whether they retained the array. The data from four animals in this group are not presented in figure 1 because they represented losses late

within portions of cell lineages not shown. All 40 CAN cells in this group were positioned normally and 38 of 40 CANs were not Ncl, indicating that they retained the array. The two CANs that were positioned normally despite having lost the array reflect the incomplete penetrance of the CAN migration defect in *cam-1(gm122)* (16).

***unc-34* cloning.** *unc-34* was mapped genetically to a small region of the left end of chromosome V, between the cosmids T03D5 and R09A1. A BLAST search of the *C. elegans* genomic sequence using sequences from the Drosophila *ena* gene detected a homolog in the same interval as *unc-34*. To determine whether *C. elegans ena* corresponded to *unc-34*, we used DNA hybridization with *C. elegans ena* probes to DNA from *unc-34* mutants as described (20). Using two different *C. elegans ena* DNA probes we detected a complete deletion of the nematode *ena* gene in *unc-34(e951)*. In addition, we determined the sequence of a 380 bp region from the N-terminus of *C. elegans ena* from eight different *unc-34* mutants. In two of the mutants we detected lesions. *unc-34(gm104)* is predicted to change amino acid 45 to an amber stop codon and *unc-34(gm114)* is predicted to change amino acid 121 from an alanine to a threonine. Taken together, these data demonstrate convincingly that *unc-34* encodes the *C. elegans* homolog of Drosophila *ena*.

RESULTS AND DISCUSSION

Identification of genes required for cell migration: I conducted two screens for mutations that disrupted cell migration in *C. elegans*. They focused on a single representative cell type, the canal-associated neurons (CANs). The CANs are a pair of bilaterally symmetric neurons that are born in the head and migrate to the middle of the animal during embryogenesis (figure 2). The CANs may function in osmoregulation and are essential for viability (16). By screening for mutants that displayed phenotypes associated with CAN defects and by screening for mutants with misplaced CANs, I identified 30 mutations that disrupted cell migration (11).

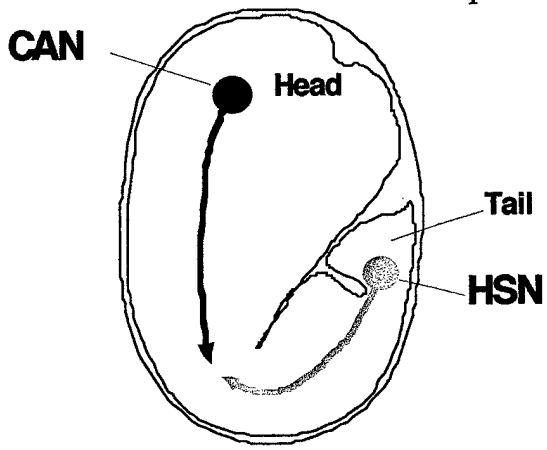

Figure 2. The CANs are born in the head and migrate to the middle of the animal during embryogenesis. The HSNs are born in the tail and migrate to a position just posterior to the CANs.

These mutations defined 14 genes required for cell migration, half of which have not been identified previously. The screens identified alleles of the six previously defined genes *epi-1, ina-1, mig-2, unc-34, unc-73* and *vab-8*, and of the seven new genes *cam-1, cam-2, syc-1, syc-2, syc-3, fam-1* and *fam-2*. The screens also identified the first mutations in the gene *ceh-10*, which encodes a homeodomain protein expressed in the CANs (21, 22). With the exception of *mig-2(gm103*sd*)*, all of the mutations are recessive and therefore likely to reduce or eliminate gene function (16).

Cell migration defects: To determine whether cell migrations other than those of the CANs were perturbed in the mutants, we examined the positions of several cells that migrate during *C. elegans* development. We examined the positions of the ALM mechanosensory neurons, the HSN motor neurons, the mesodermal coelomocytes (ccs), the M mesoblast, and the Z1/Z4 somatic gonad precursor cells, all of which migrate during embryogenesis (16, 23). We also examined the positions of the Q neuroblast descendants and the P cell descendants. The left and right Q neuroblasts and their descendants migrate during the first larval stage (24). The six bilaterally symmetric pairs of P cells cover the ventral third of newly hatched first larval stage (L1) animals. Midway through the L1, the P cells migrate ventrally and intercalate to form a row of twelve cells at the ventral midline. The cells then divide longitudinally to produce the Pn.a and Pn.p cells. The Pn.a neuroblasts divide during the L1 to produce ventral nerve cord motor neurons, and some of the Pn.p cells divide during the third larval stage to produce the hermaphrodite vulva.

Our analyses revealed that none of the mutations disrupted the migrations of Z1 and Z4, and only the *cam-2(gm124)* mutation appeared to disrupt M migration or development. We were unable to detect M in *cam-2(gm124)* mutants by Nomarski optics, suggesting that it was either severely misplaced or failed to differentiate normally.

Mutations in the nine genes *cam-1, epi-1, fam-1, fam-2, ina-1, mig-2, unc-34, unc-73* and *vab-8* affect the migrations of many cells in addition to the CANs (16). Mutations in the genes *epi-1, mig-2* and *unc-73* produce the most widespread defects, affecting all cell

migrations scored except those of M, Z1 and Z4. *cam-1* mutations perturb the positioning of the CANs, ALMs, HSNs and ccsL, cells which normally migrate to positions near the middle of the animal (16).

Axonal outgrowth is disrupted in CAN migration mutants: Cell migration and axonal outgrowth are similar processes that require many of the same genes (for example, see (25). To determine whether our mutations disrupt growth cone migrations, we examined CAN axonal morphology using the *ceh-23-gfp* reporter, HSN axonal morphology by anti-serotonin staining, and axonal morphology of the AVL, DVB, DD, and VD motor neurons by anti-GABA staining.

After the CAN cell bodies have migrated, they extend axons both anteriorly and posteriorly. To reach its normal destination in the tail, the posteriorly directed CAN axon from an anteriorly displaced CAN must extend further than the axon from a normally positioned CAN. Because posteriorly directed CAN axons in *ina-1(gm144)* and *mig-2(gm103*sd*)* mutants often extend to their normal destinations even when the cell bodies are in the head, CAN axonal defects observed in other Cam mutants probably reflect a direct requirement for gene function in CAN axonal outgrowth. Although all Cam mutations disrupted posteriorly directed CAN axonal outgrowth, the severity of such defects varied substantially. Mutations in *ceh-10* and *vab-8* severely disrupted CAN axonal outgrowth, mutations in *cam-1*, *cam-2*, *epi-1*, *unc-34*, and *unc-73* caused modest defects, and mutations in the remaining genes had little effect.

Each laterally positioned HSN cell body extends an axon ventrally along the epithelium to the ventral nerve cord, a paired bundle of axons that runs the length of the animal. Once in the cord, each axon turns anteriorly and extends along the ipsilateral axon bundle to the nerve ring, the major neuropil that encircles the pharynx. At the vulva, each HSN axon defasciculates from the ventral nerve cord and branches (13, 26). The mutants exhibited a range of HSN axonal outgrowth defects. In many mutants, the HSN cell bodies were displaced posteriorly, consistent with results obtained by Nomarski optics. The HSN axons of *fam-2*, *mig-2*, *unc-34*, and *unc-73* mutants terminated prematurely in the ventral nerve cord before reaching the nerve ring, a phenotype previously described for *mig-2*, *unc-73*, and *unc-34* mutants (14, 15). The axons of normally positioned HSNs often failed to branch at the vulva in *epi-1*, *fam-2*, *ina-1* and *mig-2* mutants.

We also examined the axonal morphology of several GABAergic neurons. The DD motor neurons are generated during embryogenesis, and the VD motor neurons are generated during the L1. Both D-type motor neurons extend anteriorly directed processes along the ventral nerve cord that branch near their anterior ends. Each branch produces a commissure that extends dorsally along the lateral epithelium. Upon reaching the dorsal midline, the commissures branch and extend posteriorly and anteriorly directed processes along the dorsal nerve cord (15). Dorsally directed commissural axons of *epi-1*, *fam-1*, *mig-2*, *unc-34*, and *unc-73* mutants often branched, stopped prematurely, or extended longitudinally along the lateral body wall, failing to reach the dorsal midline.

The ventral nerve cord often was disorganized in *epi-1*, *fam-1*, *fam-2*, *mig-2*, *unc-34*, and *unc-73* mutants. In wild-type animals, AVL and DVB also extend axons along the right ventral nerve cord (15). Anti-GABA staining reveals that all four GABAergic axons extend near each other along the right bundle. The ventral nerve cords of twelve

mutants representing six genes defasciculate, splitting into additional bundles that separate widely. This defasciculation was common in *epi-1*, *mig-2*, *unc-34*, and *unc-73* mutants and was occasionally seen in *fam-1* and *fam-2* mutants. Instead of extending as two separate axons, the HSNs of these mutants often extended both axons anteriorly as a single fascicle. This HSN defect and the GABAergic defasciculation defects seen in these mutants may result from general defects in ventral nerve cord fasciculation.

A gene that specifies cell position and polarity: Migrating cells must recognize when they have reached their proper destinations and stop migrating. One of the genes we identified, *cam-1*, appears to play a general role in this process. In *cam-1* mutants the positions of several migrating cell types are shifted anteriorly, suggesting that *cam-1* specifies the final position of multiple classes of cells.

In addition to recognizing spatial cues, the process of cell locomotion requires that a cell generate force in a single direction at its periphery. This is accomplished by the localized assembly of the protein complexes that generate the migratory force, possibly by regulating actin dynamics. Such polarization of cells is also seen in asymmetric cell division, where two daughter cells of a division differentially inherit the molecules that specify cell fate. *cam-1* also appears to play a role in polarizing cells. Six cells located laterally on each side of the animal, the V-cells, divide during the first larval stage to generate daughter cells that adopt different fates (figure 3). In *cam-1* mutants, the orientation of 10% of one of the V-cell divisions was reversed.

CAM-1 acts with a Wnt signaling protein to orient cell polarity: Multiple signaling pathways are likely to provide positional information along body axes to regulate cell position and polarity. For example, in *Drosophila* two secreted molecules, Wnt/Wg and DPP, act antagonistically to specify dorsal-ventral axis in developing imaginal disks (27).

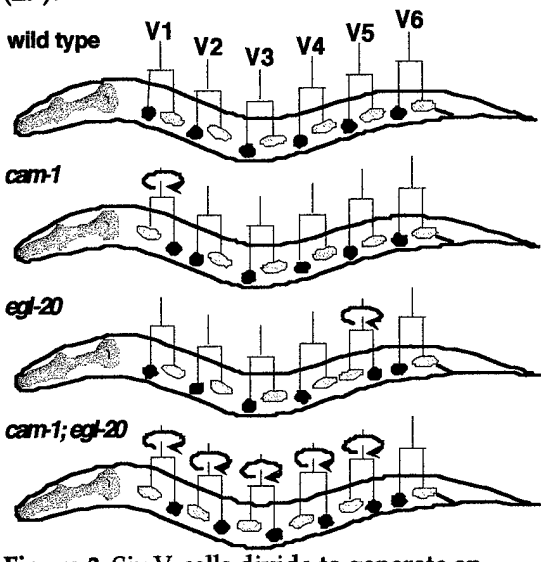

Figure 3. Six V-cells divide to generate an anterior cell that fuses with the syncitial epidermis (●) and a posterior blast cell (⌓).V1 polarity is reversed in *cam-1* mutants. V5 polarity is reversed in *egl-20* mutants. The polarity of V1 - V5 is reversed in *cam-1; egl-20* mutants.

Because of the similarities between *cam-1* and *egl-20* phenotypes, a strain mutant for both genes was generated. Analysis of the *cam-1; egl-20* double mutant revealed that the divisions of several V-cells now were reversed. These results suggest that *cam-1* encodes a signaling pathway component that acts with the EGL-20/Wnt signaling pathway to direct cell migrations and orient asymmetric cell divisions along the A-P axis.

***cam-1* encodes a receptor tyrosine kinase:** To further understand how *cam-1* acts to regulate cell position and polarity, I cloned the *cam-1* gene. *cam-1* encodes a RTK most similar to human and *Drosophila* Ror, proteins of unknown function that are expressed in neuronal tissues (5, 6, 7). The Ror proteins are closely related to the nerve growth factor receptor/Trk class of RTKs, and to MuSK, a protein involved in acetylcholine receptor clustering at the neuromuscular junction (28). Because RTKs can

function as cell surface receptors for extracellular cues, one possibility is that CAM-1 functions directly to receive cues that specify cell position and cell polarity. The similarity to MuSK raises the possibility that Ror RTKs may act to assemble proteins that polarize cells. My future studies are aimed at understanding how Ror proteins function in cell migration.

***unc-34* encodes an *enabled* homolog:** Four alleles of another gene, *unc-34*, were identified in the CAN cell migration screens described above. In addition, at least 12 other alleles of *unc-34* had been identified previously by others. *unc-34* mutants have numerous defects in the migrations of many different cell and neuronal growth cones, and have severely disorganized ventral nerve cords (15, 16). To understand how *unc-34* acts in cell and growth cone migration, we cloned the gene. We noticed that a homolog to the Drosophila *enabled* (*ena*) gene resides in the region of *unc-34* by examining data produced by the *C. elegans* genome project. Mutations in Drosophila *ena* cause severe disorganization of the central nervous system (8), a phenotype similar to that seen in *unc-34* mutants. Ena is conserved from flies to mammals and has been implicated in cytoskeletal dynamics and stability through F-actin assembly; murine Ena localizes to the cytoskeleton and binds profilin (9). The similarity of phenotypes between *ena* and *unc-34* and the presence of an *ena* homolog near *unc-34* suggested that *unc-34* might encode a *C. elegans ena* homolog.

Three results showed that *unc-34* is indeed an *ena* homologue (29). First, RNA interference with *C. elegans ena* sequences produced an *unc-34*-like phenotype. Second, genomic Southern blots of DNA from *unc-34* mutants showed that the *ena* homologue was deleted in *unc-34(e951)* mutants. Third, sequencing the 5′ end of the *ena* gene from *unc-34* mutants showed that *unc-34(gm104)* is an amber stop codon early in the gene and *unc-34(gm114)* is an alanine to threonine mutation. Our work also demonstrates that, in addition to the requirements for Ena in nerve bundle formation seen in Drosophila, *ena* plays a significant role in cell migration.

Several genes function directly in cell migration: Mutations in eight genes perturb the migrations of many cells and neuronal growth cones. Pleiotropic defects in cell migration and axonal outgrowth caused by mutations in these genes suggest that they encode proteins that function directly in cell and growth cone migrations. Recent molecular analysis of five of these genes supports this view. *epi-1* encodes a laminin α chain homolog (30). Laminin is a heterotrimeric extracellular matrix molecule that functions in cell and growth cone migrations (31). *ina-1* encodes an α integrin subunit (32). Integrins are cell surface receptors that function in cell adhesion events necessary for cell and growth cone migrations (33). *unc-73* encodes a putative regulator of Rho activity and *mig-2* encodes a Rho family member (34, 35). Rho family members function in fibroblast migration and attachment (36). As described above, *unc-34* encodes a homolog of *enabled*, which has been implicated in cell and growth cone migration. A sixth gene, *vab-8*, encodes a protein distantly related to kinesin proteins that previously have not been implicated in cell migration (37). Therefore, five of the genes encode homologs to mammalian proteins implicated in cell migration and one encodes a protein not previously implicated in cell migration. The two unidentified genes are likely to encode equally interesting proteins involved directly in cell migration.

CONCLUSIONS

We have identified 14 Cam genes that are required for *C. elegans* cell migrations. We find that these genes represent three classes: genes required for cell fate specification, genes required for multiple cell and growth cone migrations, and a single gene required for cell positioning.

Eight genes function in multiple cell and growth cone migrations. Mutations in *epi-1*, *ina-1*, *fam-1*, *fam-2*, *mig-2*, *unc-34*, *unc-73*, and *vab-8* disrupt the migrations of multiple cells and growth cones. Cell migration and axonal outgrowth defects have been described previously for *mig-2*, *unc-34*, *unc-73* and *vab-8* (14, 25, 38, 39, 40). The first *epi-1* and *ina-1* mutants were isolated on the basis of defects in epithelialization (E. Hedgecock, personal communication) and HSN migration (32), respectively.

The *cam-1* gene functions in final positioning of migrating cells. In *cam-1(gm122)* mutants the HSNs migrate too far anteriorly 72% of the time. In addition to being excessive, HSN migrations in *cam-1* mutants can also be incomplete. Moreover, ALM migrations can also be incomplete or excessive in *cam-1* mutants, although the ALM defects are less severe than the HSN defects. In summary, cells appear to migrate to their approximate, but not precise, destinations in *cam-1* mutants. These results suggest that *cam-1* acts to define the final positions of several migrating cells.

Besides specifying final cell position, *cam-1* orients cell polarity. In *cam-1* mutants, the polarity of one of the V-cells is reversed some of the time. Interestingly, *cam-1* appears to orient cell polarity in concert with *egl-20*, which encodes a Wnt signaling molecule (41). In animals mutant for both *cam-1* and *egl-20*, the polarities of additional cells are reversed.

We have cloned the *cam-1* gene, and find that it is predicted to encode a receptor tyrosine kinase of the Ror family. Ror family members have been identified in Drosophila and mammals, where they are expressed in the developing nervous system (5, 6, 7). The function of Ror proteins is unknown. Analysis of *cam-1* will provide the first indication of Ror family member function. In addition, we have cloned the *unc-34* gene, and find that it is predicted to encode a homolog of Drosophila *ena*. Experiments investigating the role of *unc-34*, and interacting proteins, in cell migration are currently underway.

Cam genes define three steps in cell migration. Analysis of the Cam mutants indicates that three genetically defined steps are necessary for cell migrations. First, cells decide to migrate. For the CANs, the gene *ceh-10*, either directly or through other genes, controls this decision to migrate. Second, cues guide the cell along its route. The gene *vab-8* guides cells and growth cones posteriorly (39, 40). Seven additional genes, *epi-1*, *fam-1*, *fam-2*, *ina-1*, *mig-2*, *unc-34*, and *unc-73*, function in multiple cell and growth cone migrations, and products of three of these genes function directly in cell and growth cone migrations as extracellular matrix molecules, cell surface receptors and signal transduction molecules. Third, extracellular cues define the final destinations of migrating cells. A cue from the CAN appears to stop the migrating HSN. In addition, the gene *cam-1* may function to define the final destinations of several migrating cells. Further molecular analysis of the genes that function in multiple cell migrations should lead to important insights into how the pathways and destinations of migrating cells are defined.

DISCUSSION IN RELATION TO STATEMENT OF WORK

Below I list the five aims proposed in my Statement of Work, and discuss specific progress made towards achieving each aim:

Aim 1: Complete the analysis of the cell migration mutants identified in the screens to determine the precise number of genes identified (months 1-4).

Progress: This work was accomplished within the time frame proposed. The results are presented in two publications (11, 16).

Aim 2: Complete phenotypic analysis of mutants to identify candidates most likely to be directly involved in cell migrations (months 1-12).

Progress: This work was completed within the time frame proposed. The results are presented in two publications(11, 16).

Aim 3: Clone two of the genes already identified as likely to be directly involved in cell migrations (months 1-24).

Progress: The two genes have been cloned. It should be pointed out that the naming of the genes has changed from the original proposal. The gene referred to as *cam-1* in the original proposal was defined by two alleles, *gm120* and *gm127*. Since the original proposal was submitted, we have discovered that these two alleles represented two unusual alleles of *ceh-10*, a *C. elegans* homeobox gene. We have presented the characterization of *ceh-10* mutations in a publication (11). We have named another gene, defined by two alleles, *gm105* and *gm122*, *cam-1*. Mutations in *cam-1* result in defects in multiple cell migrations as well as in cell polarity. To understand how *cam-1* acts to regulate cell position and polarity, we cloned the *cam-1* gene. *cam-1* appears to encode a RTK most similar to human and *Drosophila* Ror, proteins of unknown function that are expressed in neuronal tissues (5, 6, 7). The Ror proteins are closely related to the nerve growth factor receptor/Trk class of RTKs, and to MuSK, a protein involved in acetylcholine receptor clustering at the neuromuscular junction (28). Because RTKs can function as cell surface receptors for extracellular cues, one possibility is that CAM-1 functions directly to receive cues that specify cell position and cell polarity. The similarity to MuSK raises the possibility that Ror RTKs may act to assemble proteins that polarize cells. My future studies are aimed at understanding how Ror proteins function in cell migration.

The second gene, *unc-34*, has been cloned recently. *unc-34* encodes a homolog to the Drosophila *enabled* gene (29). Mutations in Drosophila *ena* result in a severely disrupted central nervous system. The murine homolog, *mena*, has been proposed to regulate actin polymerization, and thereby to regulate and direct cell migration (9). Our analysis of *unc-34* mutants demonstrates that in addition to its role in nervous system development, *C. elegans* Ena is required for proper cell migration. Our current research is aimed at understanding how Ena acts with other proteins to regulate cell migration.

Aim 4: Detemine the phenotype in the absence of all gene function (months 6-18)

Progress: We have examined the phenotypes of *cam-1(gm105* and *gm122)* in trans to a chromosomal deficiency that deletes *cam-1*. The phenotypes of mutants bearing *cam-1(gm105)* are enhanced in trans to a chromosomal deficiency, suggesting that *cam-1(gm105)* does not remove all gene function. In contrast, mutants bearing *cam-1(gm122)* in trans to a chromosomal deficiency are no more severely defective than *cam-1(gm122)* mutants, suggesting that *cam-1(gm122)* may remove all gene function. We determined the molecular lesion in *cam-1(gm122)*, which is predicted to change a glutamine to a stop

codon within the extracellular portion of the protein. We have not detected alternatively spliced forms of the *cam-1* mRNA, suggesting that only a single transcript may be produced. If this is the case, the mutation in *cam-1(gm122)* is almost certainly null.

The phenotypes of mutants bearing *unc-34* mutations in trans to deficiencies that delete the gene have been determined by our collaborators, and are no more severe than in the mutants alone, suggesting that *unc-34* mutations may be null. In addition, the molecular lesion in *unc-34(gm104)*, which is predicted to change a tyrosine to a stop codon very early in the coding region of the gene. Therefore, *unc-34(gm104)* is likely to represent a null mutation.

Aim 5: Detemine the sites of gene expression and function (months 12-24).
Progress: These experiments were proposed for the second year of funding. I have used a mosaic analysis in which some cells of an animal are mutant for *cam-1* and others are wild type to determine whether *cam-1* functions autonomously within migrating cells. The mosaic analysis demonstrated that *cam-1* is required within the migrating CAN cells. In addition, I generated a transgene that fused the entire *cam-1* gene including 3 kilobase pairs of upstream sequences to the *Aequorea victoria* green fluorescent protein gene (gfp). Gfp was inserted at the stop codon of the *cam-1* gene. This construct rescues the CAN cell migration defect of *cam-1* mutants, and therefore is likely to reflect the endogenous *cam-1* expression pattern. Animals bearing the *cam-1-gfp* transgene express GFP in much of the nervous system, as well as muscle, intestine and hypodermal cells. Expression has been detected within each of the cell types known to be affected by mutations within *cam-1*, consistent with the mosaic analysis demonstrating the *cam-1* functions cell autonomously. The expression pattern raises the possibility that *cam-1* functions cell autonomously in each of the cells affected by the mutation.

Experiments are now underway to determine the site of *unc-34* expression. These will be completed within the next several months.

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Personnel receiving pay from the negotiated effort

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